CYCLIC AMP AND COLICIN SYNTHESIS

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SUMMARY: Spontaneous synthesis of B, E, I and K colicins is largely independent of cyclic AMP.

Several reports suggest that cyclic AMP may be necessary for colicin synthesis whether spontaneous (1,2) or when increased by mitomycin-C (3,4). However, the following measurements of colicin titres in growing cultures of Escherichia coli K-12 strain PP78 (cya crp-1) which lacks adenyl cyclase and cAMP receptor protein suggest that they are never less than half the titres observed with its parent cya^+ crp^+ strain.

Methods

Bacteria. The two strains of E.coli K-12, 1100 and PP78, were kindly provided by Dr. Ira Pastan (5). Strain 1100 is HfrH thi str-s cya^+ crp^+ lac^+; its derivative, strain PP78, is HfrH thi str-r cya crp-1 and hence appears Lac^-. Each strain was made resistant to either colicin E, K or B before being grown with colicinogenic donors to prepare their respective Col^+ derivatives. ColV factors could not be tested because they are not stably inherited by an Hfr host.

Other methods. Culture media (all sterilized by heating to 121° for 15 min), lacuna counts on chloroformed samples and colicin assays on sonicated samples by radial diffusion are

described elsewhere (6). Total bacterial protein was estimated on centrifuged cells (7).

Results

In cultures carrying ColE2-P9 or ColK-235, the % colicin-containing cells as measured by lacuna counts (% LFC) may not become constant in growing cultures until 10 or more generations have elapsed (6,8). In the present experiments, cultures of Col^+ derivatives of strains 1100 and PP78 were therefore grown overnight at 37° in 2 ml unshaken YE broth without glucose, diluted 1/10,000 in the same medium, shaken at 37° and incubated for 3 hr for strain 1100, or 4 - 5 hr for strain PP78, before making the first measurements. These included the optical

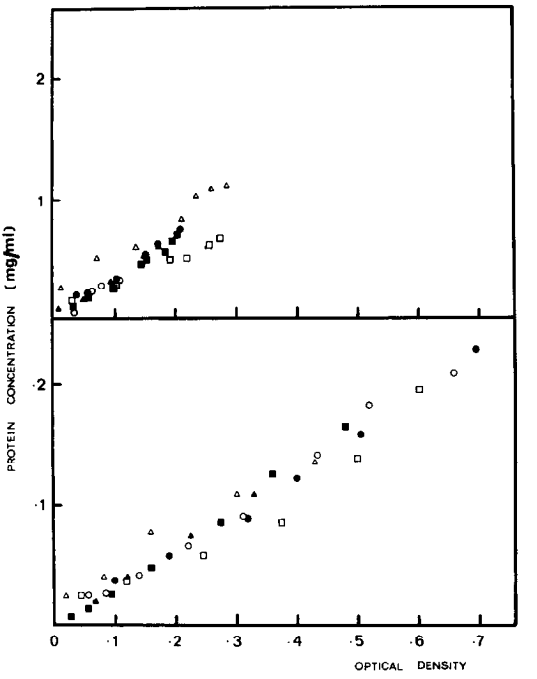

Figure 1. Relation between total bacterial protein/ml culture and O.D. for Col^+ Col^r derivatives of strains 1100 and PP78 (lower and upper graphs, respectively). Col factors: B-K98, △ ; E1-K30, ● ; E2-P9, ○ ; Ia-CT4, □ ; K-235, ■ ▲ .

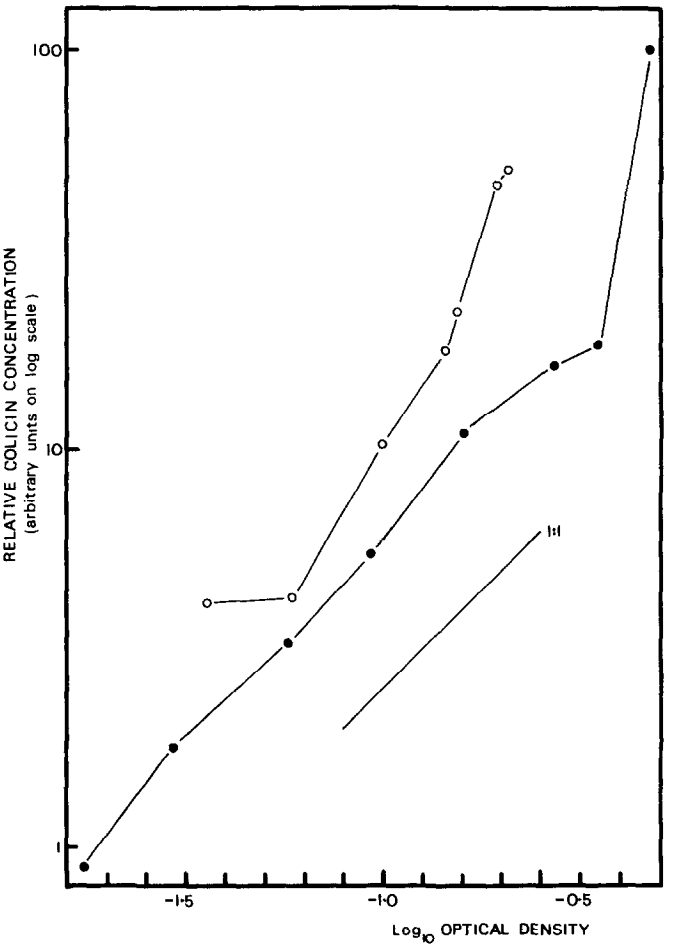

Figure 2. Relation between log relative colicin concentration /ml culture and log O.D. for ColK-235 in strains 1100 (—●—) and PP78 (—o—).

density (O.D.) at 650 nm, total bacterial protein, colicin titrations, colony counts and, with ColE and ColK factors, lacuna counts (the latter are not feasible with ColB and ColI factors: 9).

The two Col^- parental strains, and also their Col^+ derivatives, showed obvious differences since strain 1100 grew to a greater O.D. and formed larger colonies. Nevertheless, their Col^+ derivatives gave the same relationship between total bacterial protein and O.D. (Fig. 1). The relation between colicin titre

and O.D. was then determined for seven different Col factors. The results can be summarized as follows:

1. At colony counts less than 10^8/ml (O.D. = 0.1 - 0.2), a plot of colicin titre against O.D. had a slope of 1.

2. At higher colony counts, the slope for ColE$^+$ and ColK$^+$ cultures usually increased (Fig. 2), presumably because the % LFC was also increasing (6,8). No such increase in slope above 1 was apparent with the ColB and ColI cultures (Fig. 3), suggesting that the rate of colicin synthesis by

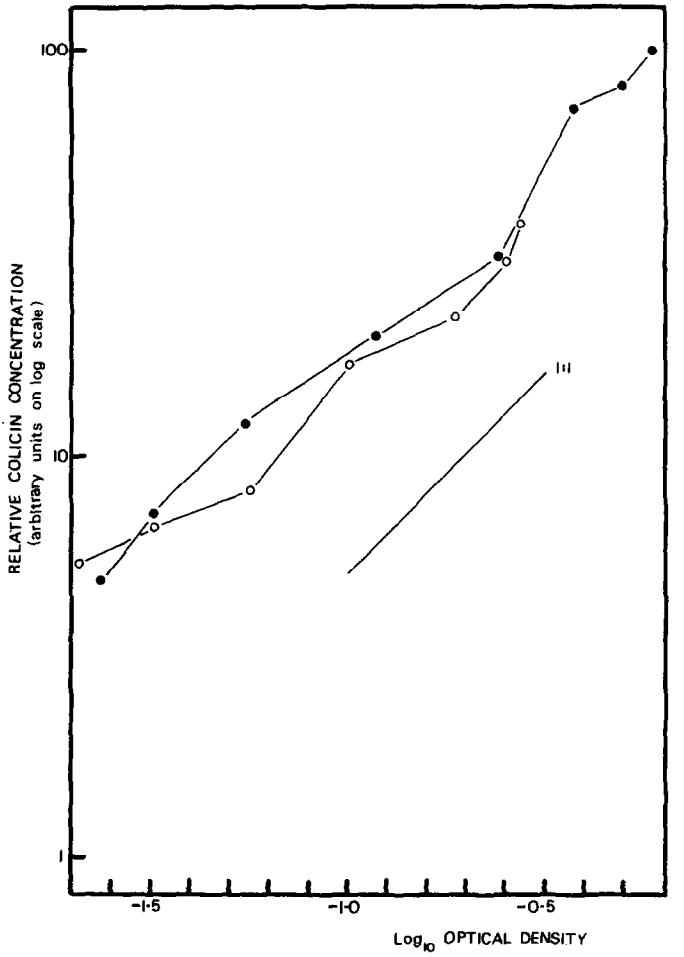

Figure 3. Relation between log relative colicin concentration /ml culture and log O.D. for ColIa-CT4 in strains 1100 (—●—) and PP78 (—○—).

these cultures was constant in this range of O.D.

3. The colicin titres produced by Col$^+$ derivatives of strain PP78 compared to those of strain 1100 were: equal (ColB-K98, ColE1-K30); less (ColB-K77, ColE1a-16, ColE2-P9, ColIa-CT4: Fig. 3); or greater (ColK-235: Fig. 2).

4. When titres produced by Col$^+$ PP78 were less than those produced by Col$^+$ 1100, the difference was no more than about two-fold (e.g. Fig. 3).

Spontaneous colicin synthesis therefore appears to be little affected by the cAMP system and, in view of the differences in growth of strains 1100 and PP78, such differences in colicin titres as were observed might well have been non-specific. The previous reports do not include measurements of colicin titres. One reported that inhibition zones around colonies were smaller with ColE2$^+$, and absent with ColE1$^+$, derivatives of strains 5336 (*cya crp*$^+$, to which strain PP78 is related) and CA-7900 (*cya*$^+$ *crp*), neither first made colicin-resistant, which were tested in the presence of glucose after only 24 hr incubation (1). The second report gives isolated lacuna counts (2) on a *cya*$^+$ *crp* strain carrying ColE2 or ColE1, which cannot be assessed without further data (6,8).

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